From the Department of Prosthetics and the Clinical Laboratory of the Royal School of Dentistry
and AB Atomenergi, Stockholm, Sweden.

INFLUENCE OF SOME DENTAL RESTORATIONS ON THE CONCENTRATIONS OF INORGANIC CONSTITUENTS OF THE TEETH

by

RUNE SÖREMARK
OVE INGELS
HARRY PLETT
KNUT SAMSAHL

There is a continuous interchange of elements between the tooth and its environment, and within the tooth itself many interrelated physical and chemical processes are involved in the absorption and distribution of these elements. A disturbance in any one of these processes can ultimately be reflected throughout the entire system, a fact of which any clinician is aware.

The purpose of the present study was to examine dentine and enamel for an increase in concentration of some inorganic constituents influenced by their presence in dental restorations.

The concentration of some trace elements in normal human enamel and dentine has been the subject of recent investigations (*Söremark & Samsahl,* 1962 a and b). The concentrations on the basis of dry weight were studied in fifteen intact first bicuspids from 14—16 year old boys and girls. The values were obtained by means of neutron activation analysis of enamel and dentine samples. Chemical group separation of the activated enamel and dentine was followed by gamma-ray spectrometric analysis.

It is an important principle that the experimental methods used for studying biological processes should not themselves cause a significant disturbance in the system under study. Be-

cause radioactive nuclides can usually be detected in amounts which are extremely minute when expressed in ordinary chemical terms, the isotope technique greatly increases the range of measurements which can be carried out without violating this condition. The choice of a particular technique for analysis of trace elements depends not only on its sensitivity and its reproducibility but also on its range and convenience. By means of radioactivation analysis many elements in minute concentrations can be detected and determined with greater sensitivity and precision than by other analytical methods. Neutron activation analysis is both sensitive and reproducible.

On the basis of the previous findings of the concentration of some trace elements in normal human enamel and dentine it seemed of interest to study the influence of dental restorations on the normal concentrations of elements in these tissues using the same analytical method.

MATERIALS AND METHODS

Investigations in vitro

Ten intact premolars, extracted for orthodontic reasons from 14—16 year old boys and girls were used in this study. Immediately after extraction the teeth were washed in saline solution for one minute and then stored in a refrigerator at 1°C until used, but no longer than for 2 days. When used the teeth were kept at about 20°C. Care was taken not to dehydrate the teeth.

Autoradiography with the radioactive gold nuclide Au^{198}

For the autoradiographic study four teeth were used in their entirety. In two teeth, ordinary class V cavities were prepared and gold inlays were made and cemented with phosphate cement. On two teeth, circumferential gold clasps were placed in contact with the intact buccal and lingual enamel surfaces.

These four teeth were stored in a saline solution at 20°C for three weeks with the gold inlays and clasps *in situ*. The gold used contained about 150 μC Au^{198} per gram gold. The radioactive gold was prepared in the neutron reactor, R 1, in Stockholm. The teeth were embedded in a special acrylic resin (Castolite) and then cut

with a diamond instrument through the fillings and clasps in longitudinal sections about 150 microns thick. The pieces of inlays and clasps in the various sections were then carefully removed. The sections, which were mounted on glass slides with doublefaced cellulose tape, were then washed in tap water for 2 hours. After drying with an air blast for 2 minutes, the sections were placed in contact with Gevaert's Dentus Rapid X-ray emulsions for exposure in order to obtain autoradiograms after a sufficient time of exposure, ordinary developing, and fixing procedures.

Quantitative measurements of the concentration of some trace elements in enamel and dentine

For these measurements six teeth were used in their entirety. On two intact premolars ordinary preparations and gold ¾-crowns were made and cemented with phosphate cement. On two teeth chromium-cobalt clasps (circumferential retainer with occlusal rest) were made; on two other teeth platinum clasps of the same type.

These six teeth were stored in a saline solution at 20°C for three weeks with their crowns and clasps *in situ*, after which time they were removed by polyethylene-covered instruments. The teeth were then washed for 2 hours in tap water. Enamel and coronal dentine were separated by chipping and breaking with polyethylene-covered instruments. Almost the total enamel and coronal dentine from each tooth was dried in an electric oven at 105°C for 48 hours. For these quantitative measurements standards in known amounts of the elements to be studied were put into separate polyethylene tubes and placed together with the unknown sample in an aluminium can for neutron activation in a flux of about 2×10^{12} neutrons $\times$ cm^{-2} $\times$ $sec.^{-1}$ for about 20 hours.

By this neutron bombardment many elements become radioactive and can thereby be identified and analyzed by measuring the radioactivity induced.

When the period of irradiation was completed, the samples were dissolved in strong HCl solution containing H_2O_2. The solution was then subjected to chemical group separation. This chemical procedure was based on a system of short ion exchangers

coupled in series for separation of the elements in eight groups. This separation was made as a pretreatment for gamma-ray spectrometric analysis in order to avoid overlapping of the radiation of the different nuclides in the spectra. The various procedures have been described by *Samsahl* (1961) and *Söremark & Samsahl* (1962 c). The activity was measured with a 256-channel gamma spectrometer. Quantitative data based on the dry weight of the enamel and dentinal samples were obtained by comparing the gamma intensity of the identified elements with those of the appropriate standards.

Investigations in vivo

Six intact premolars of the same type as those used in the studies *in vitro* described above were also used in this part of the study. The patients were males in the 16—20 year old age group.

Three weeks before the teeth were extracted an ordinary class I cavity was prepared in two of the teeth and filled with amalgam (type "68"). On two of the teeth chromium-cobalt clasps (circumferential retainer with an occlusal rest) were fitted and on two other teeth gold clasps of the same type were applied. After the three weeks and after extraction of the teeth the clasps and the amalgam fillings were removed. The teeth were then washed in tap-water for 2 hours. The enamel and coronal dentine were separated with polyethylene-covered instruments. In the further manipulations of neutron activation, chemical separation, and the measuring of the activity, the procedures described above were followed.

RESULTS

The results (*i.e.* of the studies *in vitro* as well as *in vivo*) showed in enamel and dentine high concentrations of those elements present in the restorations or clasps applied to the teeth under study. In comparison with previous studies of the normal concentrations of inorganic constituents of intact enamel and dentine from bicuspids of humans of the same age as in the present study, a significant increase of the concentrations of Ag, Zn, Au, Co, Cr, Hg, and Pt was observed.

Investigations in vitro

In the studies *in vitro* the autoradiograms showed that radiogold (Au^{198}) was taken up by the phosphate cement layer. A narrow diffuse black zone was also found in the autoradiograms corresponding to the dentinal walls of the cavity. This indicated an uptake of radiogold also in the dentine. The two teeth which had gold clasps on their enamel surfaces for three weeks also showed autoradiographically a small uptake of radiogold in the areas of the enamel surface which had been in contact with the clasps.

The quantitative measurements by means of neutron activation analysis of the whole enamel and of the coronal dentinal piece from each tooth showed a pronounced increase in the concentrations of those elements also present in the restorations and clasps.

The analysis of the concentrations of various elements in the two teeth covered with gold ¾-crowns for three weeks showed a concentration of gold in the enamel of about 1 p.p.m. based on dry weight. The normal concentration of gold in intact enamel has been found to be 0.02 p.p.m. (*Söremark & Samsahl*, 1962 a). In the coronal dentine the concentration of gold was found in the present study *in vitro* to be about 2 p.p.m. The normal concentration in coronal dentine has previously been found to be 0.03 p.p.m. (*Söremark & Samsahl*, 1962 b). No significant increase of the concentrations of other trace elements in the enamel or dentine could be observed in the gamma-ray spectra from these two teeth.

The two teeth which carried the chromium-cobalt clasps showed the following concentrations: enamel about 1—2 p.p.m. of Cr and about 1—2 p.p.m. of Co. In the dentine the concentration of these elements was low. In the teeth with the platinum clasps the concentration of Pt was about 0.1 p.p.m. in the enamel and about 0.0001 p.p.m. in the dentine. The concentrations of other trace elements in these teeth did not significantly deviate from the normal values of intact hard tissues of teeth previously studied.

The values from *all* the teeth determined in this study *in vitro* are given in Table 1 and compared to the normal concentrations of the same elements.

Table 1.
Results of studies *in vitro*.

Enamel			Dentine		
Element	Normal conc. in p.p.m.[1]) 15 teeth	Conc in p.p.m. in present study	Element	Normal conc. in p.p.m.[2]) 15 teeth	Conc. in p.p.m. in present study
Zn	276 ± 106	380—300	Zn	199 ± 78.1	216—160
Mn	0.54 ± 0.08	0.60—0.51	Mn	0.19 ± 0.06	0.22—0.26
Cu	0.26 ± 0.11	0.35—0.20	Cu	0.21 ± 0.10	0.32—0.22
Au	0.02 ± 0.01	1.2; 1.1	Au	0.03 ± 0.01	2.4; 2.1
Cr	0.005 ± 0.002[3])	1.6; 1.9	Cr	0.005 ± 0.003[3])	0.01; 0.01
Co	0.004 ± 0.001[3])	1.1; 2.3	Co	0.006 ± 0.002[3])	0.02; 0.02
Pt	—	0.08; 0.1	Pt	—	0.0001; 0.00006

1) According to *Söremark & Samsahl* (1962 a).
2) According to *Söremark & Samsahl* (1962 b).
3) Mean from three intact teeth.

Investigations in vivo

In the studies *in vivo* the neutron activation and gamma-ray spectrometric analysis of the teeth, which before being extracted had had various restorations and clasps, also showed a comparatively very high concentration of those elements which were present in the restorations or clasps which the teeth had been exposed to. No significant increase of the concentrations of other trace elements in the enamel or dentine could be observed in the gamma-ray spectra.

The teeth with the gold ¾-crowns *in situ* for three weeks showed a high concentration of gold in the enamel and dentine, about 1 p.p.m. and 2 p.p.m. respectively.

The Au-concentration of the teeth with the gold clasps was about 0.7 p.p.m. for enamel but about normal for dentine. The chromium and cobalt concentrations in the enamel of the two teeth with the chromium-cobalt clasps were about 1.5 p.p.m. for Cr and about 1 p.p.m. for Co. The corresponding figures in dentine were about 0.03 and 0.01 p.p.m.

The teeth with amalgam fillings in class I cavities showed a concentration of silver of about 25 p.p.m. in enamel and about

45 p.p.m. in dentine. The corresponding figures for Zn were 375 p.p.m. and 380 p.p.m., respectively and for Hg about 17 p.p.m. in enamel and 27 p.p.m. in dentine.

The values from *all* the teeth determined in this study *in vivo* are given in Table 2 and compared to the normal concentrations of the elements.

Table 2.
Results of studies *in vivo*.

Enamel			Dentine		
Element	Normal conc. in p p.m.[1]) 15 teeth	Conc. in p.p.m. in precent study	Element	Normal conc. in p.p.m.[2]) 15 teeth	Conc. in p.p m.) in present study
Zn	276 ± 106	436—311	Zn	199 ± 78.1	380—355
Mn	0.54 ± 0.08	0.61—0.52	Mn	0.19 ± 0.06	0.26 — 0 17
Cu	0.26 ± 0.11	0.31—0.24	Cu	0.21 ± 0.10	0.32—0.19
Au	0.02 ± 0.01	0.9;[3]) 1.1;[3]) 1.5;[4]) 0.8;[4])	Au	0.03 ± 0.01	1.6;[3]) 2.2;[3]) 0.04;[4]) 0.02;[4])
Cr	0.005 ± 0.002[5])	0.5; 1.9	Cr	0.005 ± 0.003[5])	0.02;[4]) 0.03
Co	0.004 ± 0.001[5])	0.6; 1.0	Co	0.006 ± 0.002[5])	0.01; 0.02
Ag	0.005 ± 0.002	24; 26	Ag	0.004 ± 0.002	47; 51;
Hg	—	15; 20	Hg	—	30; 24

1) According to *Söremark & Samsahl* (1962 a).
2) According to *Söremark & Samsahl* (1962 b).
3) Values from the two ¾ crown-covered teeth.
4) Values from the two teeth with gold clasps.
5) Mean from three intact teeth.

In addition to these experiments the gold concentration in enamel and dentine was studied in an upper incisor from a 16-year old boy. This tooth had been covered with a gold ¾-crown for three years. In the enamel the gold concentration was found to be 4.6 p.p.m. and in the dentine 15.7 p.p.m.

DISCUSSION

From the present investigations we may conclude that elements present in various restorative materials in contact with enamel and dentine penetrate into these tissues. The present investigations *in vivo* and *in vitro* clearly seem to indicate this.

The concentration in the dental hard tissues will consequently increase for those elements which also are present in the restorations. The present study cannot show the rate of transport of ions or molecules from the restorative material into the teeth. However, it would seem that this transport continues for a long period of time. Probably the rate of uptake of elements in the hard tissues of the teeth is exponentially in agreement with the rate of enamel uptake of various topically applied ions. The time for reaching the steady state would also be interesting to know. According to *Robertson* (1957) the term "steady state" denotes the concentration of the certain element in a tissue (here enamel and dentine) at which the rate of removal of the element is equal to the rate of replacement of new ions or molecules of the element in question.

Nearly all of the enamel and the entire coronal dentine were subjected to neutron activation and gamma-ray spectrometric analysis in the present study. This means that the values obtained for the concentrations of the various elements represent a mean concentration of the element in question in the whole sample from each tooth. Consequently, the present study cannot show the concentration of the element in the surface layer of the enamel and dentine in immediate contact with the metal restorations. However, it can be expected that the surface in contact with the restorations and clasps had a very high concentration of the elements in question in comparison with the mean concentration of the same element in the whole sample under study. The autoradiograms are offered as evidence for this assumption. It will be noted that in the present study using the very sensitive analysis methods for quantitative measurements, grinding layers or sections from the hard tissues by means of various instruments would probably have contaminated the samples.

A surprisingly good agreement was found between the values obtained in the studies *in vitro* and *in vivo*.

For further discussions of the concentrations of trace elements in the hard tissues of the teeth see the reviews by *Leicester* (1953) and *Jenkins* (1960).

SUMMARY

To obtain a better impression of just how vividly the dental hard tissues interact with their environment, studies were carried out *in vitro* and *in vivo* on intact human teeth. The teeth were analysed after having been restored with gold inlays, silver amalgam, and gold veneer crowns, or after having had gold or chromium-cobalt clasps in contact with their enamel surfaces for given periods of time.

To determine if and where penetration of these metals into the tooth substance had occurred, two analytical procedures were employed, (1) the autoradiographic method using the gold nuclide Au^{198} in the clasps and restorations; (2) neutron activation and chemical group separation in combination with gamma-ray spectrometry.

In all of the experimental teeth were the concentrations of the elements of the clasps and restorations markedly higher than in teeth not having been exposed to similar conditions.

RÉSUMÉ

INFLUENCE DE QUELQUES RESTAURATIONS DENTAIRES SUR LES CONCENTRATIONS DES COMPOSANTS INORGANIQUES DE DENTS

Pour se faire une meilleure idée justement comment les tissus durs dentaires collaborent à leurs environs des études *in vitro* et *in vivo* ont été faites de dents intactes d'homme. Les dents ont été analysées après restauration par introduction d'or, amalgame d'argent, couronnes d'or ou après position de crochets d'or ou chrome-cobalt en contact de leur surfaces d'émail pendant certaines périodes de temps.

Pour déterminer si et ou la pénétration par la substance dentaire de ces métaux avait eu lieu, deux procédés analytiques ont été employés; 1) la méthode autoradiographique par la nuclide d'or Au^{198} aux crochets et restaurations; 2) activation de neutrons et séparation chimique de groupes en combinaison de spectrométrie de rayons gamma.

De toutes les dents expérimentales les concentrations des éléments des crochets et des restaurations étaient considérablement plus hautes que celles des dents pas exposées aux conditions similaires.

ZUSAMMENFASSUNG

DER EINFLUSS EINIGER DENTALER RESTORATIONEN AUF DIE KONZENTRATION DER ANORGANISCHEN BESTANDTEILE DER ZÄHNE

Um einen besseren Einblick zu gewinnen, in welchem Umfang die Hartgewebe der Zähne von ihrer Umgebung beeinflusst werden, wurden Untersuchungen an gesunden menschlichen Zähnen ausgeführt *in vitro* und *in vivo*. Die Zähne wurden analysiert, nachdem sie mit Gold-Inlays, Silberamalgamfüllungen oder Goldkronen versehen worden waren oder nachdem Gold- oder Chrom-Kobaltklammern eine bestimmte Zeit mit ihrer Schmelzoberfläche in Kontakt gewesen waren.

Um zu bestimmen, ob und wo die betreffenden Metalle in die Zahnsubstanz eingedrungen waren, wurden 2 analytische Untersuchungsverfahren verwendet: 1) Die autoradiographische Methode, wobei das radioaktive Au^{198} in den Klammern bezw. Füllungen zur Anwendung kam, 2) Neutron-Aktivierung und chemische Gruppentrennung in Verbindung mit Gammastrahlen-Spektroskopie.

In allen so untersuchten Zähnen war die Konzentration der Stoffe aus den Klammern resp. Restorationen markant höher als in Zähnen, die nicht solchen Berührungen ausgesetzt waren.

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